

# INCIDENCE, SIZE AND SPATIAL STRUCTURE OF CLONES IN SECOND-GROWTH STANDS OF COAST REDWOOD, *SEQUOIA SEMPERVIRENS* (CUPRESSACEAE)<sup>1</sup>

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The ecology and evolutionary potential of coast redwood (*Sequoia sempervirens*) is significantly influenced by the important role clonal spread plays in its reproduction and site persistence. In nine second-growth stands, amplified fragment length polymorphisms (AFLPs) were used to identify redwood clonal architecture. Clones (multistem genets) dominated sites by representing an average of 70% of stems measured, ranging in size from two to 20 stems. As a result, a relatively small number of genets can monopolize a disproportionate amount of site resources, are more likely to persist over time, and have greater on-site genetic representation. Clones were not limited to fairy-ring structures, but consisted of a wide range of shapes including concentric rings, ring chains, disjunct, and linear structures. Between-ramet distances of up to 40 m were measured, indicating that clonal reproduction is not limited to basal stump resprouting. Clonal structure in second-growth stands was similar to earlier reports from old growth, emphasizing the importance of site persistence and long-term, gradual site development. Smaller ramet numbers per genet in old growth is probably due to local within-genet self thinning. Management and conservation of redwoods will benefit from a better understanding of the dynamics and structure of clonal spread in these forests.

**Key words:** AFLPs; clonal spread; genet; molecular markers; ramet; *Sequoia*; spatial structure; woody plant.

Asexual regeneration through sprouting from vegetative material is a common phenomenon in plants (Stuefer et al., 2002), and many community types are dominated by such clonal species (van Groenendael and de Kroon, 1990; Klimeš et al., 1997; Oborny and Kun, 2002). Commonly, clonal plants reproduce both sexually, by the production and germination of seeds, and asexually, by sprouting from roots and shoots. Despite the fact that both means of reproduction play an important role in the ecology and evolution of plant species (de Kroon and van Groenendael, 1990; Fischer and van Kleunen, 2002; Pan and Price, 2002), the majority of research involving plant population dynamics has focused on issues of sexual reproduction (fertilization, seed dormancy, longevity, morphological characteristics, dispersal and germination requirements, etc.). This is reflected in the emphasis such reproduction is given in most ecological and evolutionary theories and models. Yet, in many species the persistence of existing plant genotypes through clonal spread can be the more important avenue to plant population regeneration and maintenance (Bond and Midgely, 2001).

The ecological significance of clonal spread arises from the benefits it confers to the plant. Some benefits include effective resource acquisition as a result of foraging (Oborny and Cain, 1997), division of labor (Alpert and Stuefer, 1997), and resource sharing (Jónsdóttir and Watson, 1997). Others include better survival relative to seedlings of offspring in new and sometimes difficult environments (Peterson and Jones, 1997), reduced population turnover and dependence on seeds (Bond and Midgely, 2001), and risk evasion on a genet scale (Eriks-

son and Jerling, 1990). Evaluated ecologically, these benefits can result in high levels of productivity, in competitive advantage for site colonization and persistence, and in an ability to survive a wide range of disturbances.

Through its influence on plant fitness, clonal spread also has important evolutionary implications. Clonal spread is the vegetative establishment of genetically identical ramets that allow the persistence of a genet long after the original ortet is gone. There may be no limit to the number of ramet generations that can be generated over time within a clone, and each additional ramet adds to the overall fitness of the genet through survivorship, future seed production, and subsequent ramet production (Fischer and van Kleunen, 2002; Pan and Price, 2002). In this way, clonal spread can influence within- and among-population genetic structure, effective population size, metapopulation dynamics, natural selection, and possibly even the evolution of geographic range (Eckert, 2002).

Despite the potential importance of clonality and the fact that many woody plants are capable of vegetative regeneration and clonal spread through sprouting (Peterson and Jones, 1997), surprisingly few clonal studies have focused on trees (Barsoum, 2002), and sprouting “has received very little attention in models and theories of forest succession or forest diversity” (Bond and Midgely, 2001, p. 49). Among woody plants, the little work that has been reported has focused primarily on angiosperms.

Redwoods (*Sequoia sempervirens* (D. Don) Endl.) are one of the most productive timber types of the United States (Oliver et al., 1994) and provide an important supply of high-quality wood fiber. Yet, beyond the fact that coast redwood is exceptional for a gymnosperm in its ability to readily resprout and grow clonally after disturbances such as fires, floods, landslides, and harvesting (Rydelius and Libby, 1993; Rogers, 1994, 2000), very little is known about the spatial structure of redwood clones, the relative importance of clonal vs. nonclonal reproduction, or the variables that influence clonal spread.

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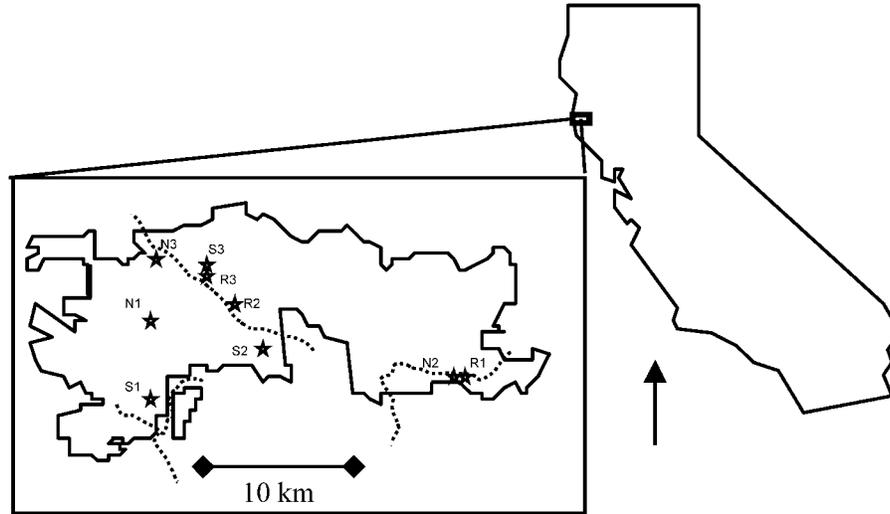


Fig. 1. Sample sites of *Sequoia sempervirens* in the Jackson Demonstration State Forest, north coast of California, USA. Sites are represented by stars (river = R1, R2, R3; north-facing = N1, N2, N3; south-facing = S1, S2, S3) and rivers are marked as dotted lines.

In a study of old-growth stands using allozyme markers, Rogers (2000) found that a significant number of redwood stems were not genetically distinct individuals, but were ramets of larger genets, and thus resulted from cloning and not from reproduction by seed. There are no similar studies of second-growth redwood forests despite the fact that in 1990 as much as 96% of those lands in commercial redwood production available for harvest were made up of young or second-growth stands (California Department of Forestry and Fire Protection, 1990).

If we are to properly understand the ecology and evolutionary potential of redwoods, and clonal woody plants in general, it is essential to further explore the structure, dynamics, and significance of clonal spread in these forests. In the context of management and conservation, for example, this would result in more accurate estimates of minimum viable population size. This is the first major study of clonal spread in redwoods using molecular genetic techniques.

It has been suggested that resprouters tend to be shorter than reseeders (Midgley, 1996); this is evidently not true when considering coast redwoods. Our primary goals were to evaluate the importance of clonal spread in second-growth redwood

stands and explore the local structure of redwood clones. If clonal spread is important in redwood stands, Bellingham and Sparrow (2000) predict that it should be influenced by disturbance frequency and site productivity. Therefore, we were also interested in determining if variability in broad site characteristics (i.e., aspect, disturbance levels) affects the importance of cloning and clone structure at a stand scale.

MATERIALS AND METHODS

The study was conducted in the Jackson Demonstration State Forest (Fig. 1) because of its combination of historic intensive management and the availability of mature second-growth stands. A total of nine sites were selected (Table 1). Three sites each were selected on north-facing slopes (N), south-facing slopes (S), and on floodplains adjacent to rivers (R). These broad categories were chosen in order to investigate possible differences resulting from variation in solar availability and moisture levels (north- vs. south-facing slopes) and variation in disturbance regime (fire more common in the upland sites vs. flooding in the riparian sites). Sites were widely distributed across the forest, were at least 500 m from each other, and were pure stands of redwood estimated to have been last cut 50–70 yr ago.

On each site, up to 45 adjacent trees over 10 cm in diameter at breast height (dbh) were mapped, tagged, measured for dbh, and sampled for DNA.

TABLE 1. Site characteristics for *Sequoia sempervirens* stands in Jackson Demonstration State Forest, north coast of California, USA.

Site	Area sampled (m <sup>2</sup> )	No. stems sampled	Stand density (no. stems/m <sup>2</sup> )	Mean stem diameter (cm) (SE)	Surrounding forest canopy character	Site moisture	Solar availability
<b>Riparian</b>							
R1	1000	37	0.037	41.4 (3.8)	Continuous	Hydric–Mesic	Moderate–Low
R2	2600	45	0.017	41.6 (2.3)	Continuous	Hydric–Mesic	Moderate–Low
R3	1550	42	0.027	41.6 (4.3)	Continuous	Hydric–Mesic	Moderate–Low
<b>North-facing</b>							
N1	1600	42	0.026	28.7 (2.0)	Continuous	Mesic	Low
N2	4000	31	0.008	54.6 (6.6)	Continuous	Mesic	Low
N3	1500	43	0.029	40.6 (3.8)	Continuous	Mesic	Low
<b>South-facing</b>							
S1	1000	38	0.038	44.7 (6.4)	Patchy	Mesic	Moderate–High
S2	1700	37	0.022	48.0 (2.5)	Patchy	Mesic	Moderate–High
S3	1000	41	0.041	43.9 (3.3)	Patchy	Mesic	Moderate–High

Sampling included clumped and forked stems that met the size requirement at forking point. With a target of up to 45 trees sampled per site, different stand densities resulted in a variation in total site areas (Table 1). In a few instances we were unable to collect leaf material due to the extreme height or canopy structure of a tree. Mean stem diameters were measured for comparison in parallel plots of the same size outside the sampled stands and no significant differences were found.

**DNA extraction**—DNA was extracted according to the Cullings (1992) modification of Doyle and Doyle (1987). DNA concentrations were established by electrophoresis on agarose gels and comparisons with lambda standards.

**AFLP analysis**—The amplified fragment length polymorphism (AFLP) method developed by Vos et al. (1995) was performed with the following modifications: Restriction digestion and ligation were performed simultaneously in a 50  $\mu$ L solution containing 250 ng of genomic DNA, 5 units (U) of *EcoRI*, 5 U of *MseI*, 1  $\times$  restriction-ligation buffer, 1 U T4 DNA ligase, 0.2 mmol/L ATP, 1.0  $\mu$ mol/L *MseI* adapter, and 0.1  $\mu$ mol/L *EcoRI* adapter. The restriction-ligation reaction was incubated for 4 h at 37°C, then diluted to 200  $\mu$ L in Tris-EDTA buffer. Pre-amplification was performed in a 25  $\mu$ L solution containing 2.5  $\mu$ L of diluted restriction-ligation product, 0.2 mmol/L dNTPs, 0.3  $\mu$ mol/L of each primary amplification primer, 1  $\times$  polymerase chain reaction (PCR) buffer, and 0.5 U *Taq* polymerase. For the primary amplification primers, *EcoRI* primer was identical to the adapter sequence, whereas the *MseI* primer had an extra “C” as a selective nucleotide. The PCR reaction was performed on a Techne Genius thermocycler for 28 cycles using the following cycling parameters: 30 s at 94°C, 60 s at 60°C, and 60 s at 72°C. The primary amplification product was then diluted to 250  $\mu$ L in Tris-EDTA buffer. Selective amplification was performed in a 25  $\mu$ L solution containing 6.25  $\mu$ L diluted primary amplification product, 0.2 mmol/L dNTPs, 0.06  $\mu$ mol/L *EcoRI* fluorescent selective primer, 0.3  $\mu$ mol/L *MseI* selective primer, 1  $\times$  PCR buffer, and 0.5 U *Taq* polymerase. We prescreened 32 selective primer pairs and chose four pairs that were reproducible and adequately polymorphic (>100 polymorphic loci per primer) for this study (*MseI*-CCAC/*EcoRI*-ACG, *MseI*-CACA/*EcoRI*-AC, *MseI*-CCAA/*EcoRI*-CCC, and *MseI*-CCAC/*EcoRI*-CAC). The selective PCR reaction had two cycle sets: 13 cycles of 30 s at 94°C, 30 s at 65°C (annealing temperature was lowered 0.7°C at each cycle), and 60 s at 72°C, followed by 18 cycles of 30 s at 94°C, 30 s at 56°C, and 60 s at 72°C. Fingerprint data were obtained by running the amplified samples on an ABI Prism 3100 DNA sequencing system (PE Applied Biosystems, Foster City, California, USA), including a size standard in each lane, using PE Applied Biosystems protocols. Band scoring was completed with the Genescan and Genotyper software (PE Applied Biosystems).

**Clone identification**—Because of the very large numbers of loci that can be detected, the AFLP method can be a very sensitive fingerprinting technique with enormous potential for assigning individuals to clones. However, choice of loci is critical; too few or low levels of polymorphism may result in lack of resolution of genetically distinct, but closely related individuals, and too many or excessive levels of polymorphism may reveal confusing within-genet differences. To minimize these risks, we selected loci that were polymorphic within sets of full sibs and developed a statistical method for clone identification (Douhovnikoff and Dodd, 2003). Using this methodology, three control sets of four known full sibs and five control sets with six clonal replicates were used to calibrate a clone identification threshold. Briefly, clonal pairwise Jaccard similarities and full sib pair Jaccard similarities from AFLP fingerprints resulted in overlapping frequency distributions. The threshold similarity (0.974) at, or above which, two sample fingerprints were considered to belong to the same clone was determined by equating the lower tail of the clonal distribution (minimum of 0.969) with the upper tail of the full sib distribution (maximum of 0.977). The full sib and clonal replicates used to determine this threshold were 24-yr-old trees planted at the University of California Russell Reservation Experimental Field Station, Lafayette, California.

**Clonal importance**—To assess the relative importance of clonal spread on each site, sample percentage distinguishable (PD) values were calculated according to Ellstrand and Roose (1987). PD values are calculated as the number of genets identified divided by the total number of stems genetically sampled. This metric of clonal diversity measures what percentage of all trees sampled is genetically distinct.

The size of genets was measured in two ways. First, the total number of ramets per genet was tabulated to evaluate unit size. Second, aggregate basal areas per genet were calculated to serve as a surrogate for biomass.

**Clonal spatial structure**—To test for spatial clustering of clones, we compared the clonal structure based on our threshold approach with the spatial pattern of samples at each of the field sites by means of Mantel tests. For each of the field sites, Mantel *R* correlations were computed between Euclidean distance matrices calculated from the point coordinates for all sampled individuals and binary matrices, in which pairs of individuals assigned to the same clone were given the value 1 and all non-clone pairs were given the value 0. Significance of the correlations was tested by 1000 random permutations. Mantel *R* correlation coefficients were *Z* transformed to produce a parameter with an approximate normal distribution (Sokal and Rohlf, 1995) and homogeneity among the *Z*-transformed Mantel *R* correlations for all site types was tested using a chi-square distribution.

## RESULTS

**The incidence and size of clones**—A total of 356 redwood stems were genetically sampled over all nine sites. For each stem, a genetic fingerprint was produced, allowing for its identification and comparison to other stems. When single stem genets (SSGs) were distinguished from multistem genets (clones), a total of 35 clones (24% of all genets) and 113 SSGs were identified on the nine sites sampled (Table 2). In general, clones were large, ranging in size from two to 20 ramets with a mean of 6.7 ramets per clone (SE = 0.75).

The PD values are an inverse measure of the importance of clonal spread on a site. The smaller the value, the greater is the proportion of stems that are replicate ramets of clones. In this study, PD values were as low as 0.19, with an all-site mean of 0.43 (SE = 0.05). On average, 32% of the stems on a site were SSGs. The remaining 68% were ramets of an average of four clones per site.

No significant difference was detected in stem basal area between individual clone ramets (mean = 0.18 m<sup>2</sup>) and SSGs (mean = 0.20 m<sup>2</sup>) within sites (*F* ratio = 0.36, *P* = 0.55). However, on a whole genet basis, mean clone basal area per site, 1.36 m<sup>2</sup> (SE = 0.26), was more than six times greater than mean SSG basal area per site (0.20 m<sup>2</sup>, SE = 0.03). The SSGs represented an average of 31% (SE = 0.05) of total basal area of all trees sampled (Fig. 2). Among multiple stemmed genets and SSGs, a full range of ramet diameters was found. This may have been a result of differential development due to resource availability and competition or to a difference in ramet initiation time.

**Clone structure**—In most cases, members of clones formed partial circles surrounding large stumps, in a “fairy-ring” structure. However, in several cases not all stems in a fairy ring were identified as part of the same clone (Fig. 3d). Also, clones were not limited to a single ring structure. In fact, a wide range of structures was detected, including figure eights or chains of multiple overlapping rings, each centered on a different stump as found on site S2 (Fig. 3a); concentric rings within larger rings as found on site S1 (Fig. 3b); disjunct clones with no clear pattern, or even stumps from which they

TABLE 2. Data for nine sites across three *Sequoia sempervirens* site types, in Jackson Demonstration State Forest.

Site	No. genets	No. clones	No. SSG <sup>a</sup> (percentage stem total)	Basal area SSG (percentage total)	PD value	Mantel
<b>Riparian</b>						
R1	9	6	3 (8%)	14%	0.24	-0.41
R2	17	6	11 (24%)	30%	0.38	-0.42
R3	21	2	19 (45%)	34%	0.50	-0.11
<b>North-facing</b>						
N1	25	3	22 (52%)	44%	0.60	-0.14
N2	11	3	8 (25%)	30%	0.35	-0.45
N3	26	3	23 (53%)	45%	0.60	-0.34
<b>South-facing</b>						
S1	10	4	6 (16%)	20%	0.26	-0.63
S2	7	4	3 (8%)	9%	0.19	-0.59
S3	22	4	18 (54%)	57%	0.54	-0.22
Totals	148	35	113 (32%)	31%	NA	NA

radiate as found on site R1 (Fig. 3c); and distantly disjunct clones with a small satellite cluster at distances as great as 40 m from the larger ring as found on site N2 (Fig. 3d).

Mantel correlations between spatial distances among stems on a site and assignment to clones ranged from -0.11 to -0.63. All correlations were negative, indicating a tendency for stems of the same genet to be clustered. The chi-square tests revealed no significant differences among site types in the Z-transformed Mantel *R* correlations, suggesting no significant difference in spatial structure among site types. How-

ever, when all sites were considered independent of type, a positive correlation was found between PD values and Mantel *R* correlations ( $R^2 = 0.674$ ,  $P = 0.006$ ), indicating that as the importance of clonal spread on a site increased, within-clone clustering of ramets increased. In other words, each additional ramet tends to be located near the other ramets in a genet.

**Variation in clone structure by site type**—The only significant difference in clonal structure detected among site types was maximum between-ramet distances, which were signifi-

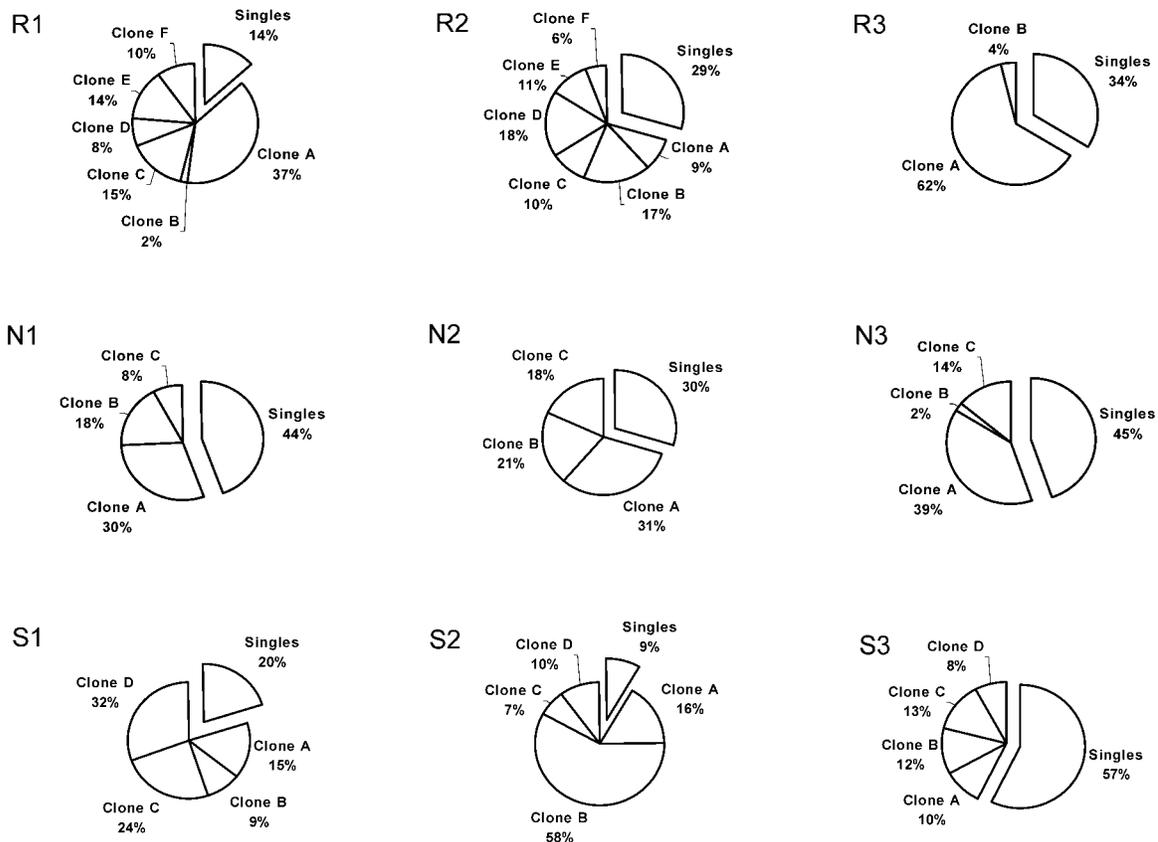


Fig. 2. Percentage of total basal area represented by each clone and all single stem genets (singles) for each *Sequoia sempervirens* site (river = R1, R2, R3; north-facing = N1, N2, N3; south-facing = S1, S2, S3) in the Jackson Demonstration State Forest.

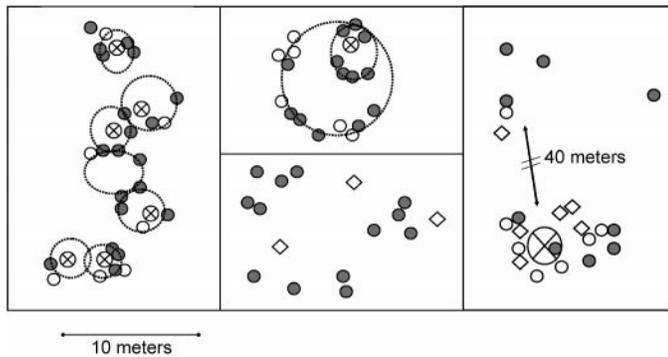


Fig. 3. A selection of four genets that demonstrate the range of *Sequoia sempervirens* clonal structures found in the Jackson Demonstration State Forest, north coast of California, including (a) chains of overlapping rings on site S2, (b) concentric rings on site S1, (c) disjunct stems on site R1, and (d) distantly disjunct stems on site N2. Solid circles represent ramets from a single clone. Open circles represent trees not sampled, open diamonds represent single stem genets, crossed circles represent stumps, and dotted circles outline perceived fairy-rings.

cantly greater on the north-facing slopes ( $P = 0.014$ ). This was not due to a small-scale density difference, but appeared to be due to a more common occurrence of disjunct ramets. Ramet clusters were found to be as far as 40 m from each other on these north-facing slopes. Maximum between ramet distances were positively correlated with the number of stems in a clone ( $R^2 = 0.290$ ,  $P = 0.002$ ) so that clones with larger numbers of ramets tended to have a greater overall footprint.

A weak but suggestive negative correlation was found between mean stem diameters at a site and Mantel  $R$  correlations ( $R^2 = 0.389$ ,  $P = 0.072$ ), implying that increased clustering of clonal ramets was associated with larger mean stem diameters at the site. Accordingly, a weak negative correlation between site PD value and mean stem diameter at a site was also detected ( $R^2 = 0.347$ ,  $P = 0.095$ ), suggesting that as the importance of cloning at a site increased, mean stem diameters at the site also increased. In summary, clonal importance, stem clustering, and stem diameters appear to be positively correlated.

## DISCUSSION

**Importance of clonal spread and clone structure**—Our molecular analyses reveal that clonal spread is an important mode of regeneration in redwood stands. The PD values measured are similar to those of sandbar willows (*Salix exigua* Nutt.) (Douhovnikoff and Dodd, 2003) and many clonal non-woody plant species such as bracken fern (*Pteridium aquilinum* (L.) Kuhn) (Parks and Werth, 1993) and *Vaccinium stamineum* L. (Kreher et al., 2000). The distribution of genet sizes is skewed by several SSGs and a few disproportionately large clones with as many as 20 stems. As a result, 2–6 genets can contain the great majority of stems on sites ranging in size from 1000 m<sup>2</sup> to 3000 m<sup>2</sup>. On these sites, the numbers of distinct genets were only about one-sixth of the total number of stems. The potential for high numbers of identical genotypes is commonly overlooked in ecological studies, but our results underline the need to take this mode of regeneration into account in clonal species such as redwood.

We are unable to determine if a stem was the direct result of a seeding event. Even an SSG may be the last in a series

of clonal events. On the other hand, it is possible to determine the minimum proportion of ramets that have resulted from clonal events. In this study, we found that on average 59% of the stems at a site resulted from clonal spread, with a high of 81% and a low of 40%. These are minimum values, and it is possible that 100% of the stems are clonal copies of preexisting stems that no longer exist.

The skew in ramet number per genet is likely a result of the unevenness of disturbance events that open a window of opportunity for sprout release. For example, a disturbed tree (windfall, burned, cut, etc.) may clear an opening and promote sprouting at its base and possibly along its fallen stem resulting in a large number of ramets in a particular clone relative to undisturbed genets. Regardless of the cause of this imbalance it is most significant that a few genets monopolize a site as large clones. This has the following interesting ecological and evolutionary implications:

(1) *Resource use*—Site resource use per genet is likely to be skewed toward larger clones. By virtue of sheer ramet numbers, if all ramets draw on local resources equally, the genet with the most ramets gets most of the resources that are available. This is compounded by the fact that if the ramets in a clone remain integrated, resources can be shared across the genet. As a result, large redwood clones not only have a proportionately larger footprint in terms of resource use, but they may also be taking advantage of economies of scale and greater access to resources. Future work on the integration of redwood clones and the extent and dynamics of resource sharing is necessary to determine the larger scale physiological importance of cloning in redwood.

(2) *Site persistence*—Large redwood clones may have important long-term advantages in maintaining site representation and dominance. This possibility is based on the following reasoning: (A) Clones are genetic replicates of locally successful genotypes giving them a competitive edge over newcomers that are subject to the trial and error involved in sexual reproduction. (B) A large proportion of redwood seeds are sterile at the time of dissemination (Olsen et al., 1990). Those that are not have a very short period of viability (Fritz and Rydellius, 1966), and the few seeds that do germinate are extremely susceptible to damping off (Hepting, 1971). On the other hand, less seasonality is associated with sprouting, allowing for a quick response to resource availability. Clones have access to an already established root system, grow much faster than seedlings, and are not susceptible to damping off (Olsen et al., 1990). (C) In many cases, stochastic events act upon clone ramets independently. As a result, the risk of mortality to a genet can be spread among its many ramets (Cook, 1983; Eriksson and Jerling, 1990). With more ramets, a redwood genet is more likely to survive stem-removing disturbances. (D) Ramet clumping diversifies stem location and can produce local characteristics that buffer some ramets from damage (Peterson and Jones, 1997). For example, uphill redwood ramets tend to be disproportionately damaged when fuels that build up on the uphill side of clones burn in a fire, whereas the downhill stems are buffered from damage. (E) Without a biological limit to the number of ramet generations over time that can be produced vegetatively, a successful redwood genotype is locally persistent and potentially immortal.

(3) *Greater genetic representation*—Large redwood genets have greater genetic representation on a site with more stems, pollen, and seeds—all important factors driving evolutionary potential. When regeneration by seed is possible, larger clones have a greater likelihood of being a genetic contributor to the cohort.

“Although clonal plants generally sprout, only a small fraction of woody sprouters are clonal and capable of vegetative spread” (Bond and Midgley, 2001, p. 45). The important distinction here is that sprouting on a local scale emphasizes stem replacement, whereas clonal spread emphasizes lateral ramet multiplication in addition to stem replacement. Our results show that redwoods are an example of the latter. We identified many distinct clonal structures, demonstrating great variability in how redwood clones spread. The well-recognized fairy rings were a standard unit of clonal structure typical of the local sprouter type, but at a larger scale, clones showed a wide range of shapes with large numbers of ramets that were unlikely to thin to a single replacement stem over time. For example, ramets from the same clone were found to be up to 40 m apart from each other and, without the limitations of our sample size, may prove to be even more widely dispersed.

Little is known about how redwood clonal spread occurs beyond local basal sprouting. While fairy rings, concentric circles, and figure eights or longer chains can be explained by repeated basal sprouting of new ramets, it is still unclear how disjunct structures come about. Rogers (2000) suggests that this may be the result of trees falling, branches being buried by the impact, and finally resprouting at distances equivalent in some cases to the average height of these trees. Olsen et al. (1990) point out that redwoods are able to sprout anywhere along the stem and that this could also result in the establishment of distant sprouts along fallen trees. Another possibility is that ramets may be produced from disturbed existing root systems (Weber, 1990; Lavertu et al., 1994). In our second-growth stands, more complex patterns of clonal structure may have resulted from site disturbance during harvesting and the movement of soil and slash. An example of this may be the disjunct and somewhat chaotic pattern found on site R1 (Fig. 3c). This is an important area for further research.

We were unable, from our data, to determine if redwood clonal spread is of a phalanx or guerilla type. While Mantel test results suggested a phalanx structure with well-clustered ramets, mapping revealed instances of well-spread ramets more typical of a guerilla structure. It is most likely that redwoods combine phalanx and guerilla types of clonal spread. This emphasizes the need to complement tests of association, such as Mantel tests used here, with direct mapping of clonal structure.

*Old growth vs. second growth and site variability*—Comparison of our results with clonal structure of old-growth stands (Rogers, 1994, 2000) provides insight into how clonal structure may change as stands develop through time. These comparisons need to be treated with some caution as isozymes were used for the identification of ramets in old-growth stands. These markers are less efficient for this purpose than the AFLPs used in our second-growth work (Smulders et al., 2002).

In general, the results from old-growth sites were very similar to those from second-growth sites, both having similar structures, such as multiple genets in a fairy ring, disjunct ramets, and complex clone configurations. In a clonal forest,

a harvest appears to be a one-time clearing of ramets, leaving potential sprouting sites (stumps, root systems, etc.) that are the result of site history that predates this event. As a result, the long-term development of large-scale clonal structure may be more important than the short-term effects of harvesting. In fact, a harvest may simply resemble another in a long series of disturbances (fire, flood, etc.) that the genets have survived over time.

The most significant difference between second-growth redwood forests and old growth forests was the greater number of ramets per genet found in the former. Second growth stands had a maximum of 20 ramets per clone and the site with the greatest mean number of ramets per genet (5.29, including single stem ramets), whereas the old growth stands had a maximum of six ramets per clone, and site mean values of less than two. Presumably, gradual self-thinning has occurred within the denser clumps of ramets, resulting in fewer ramets per genet surviving through time. In the absence of disturbance and new resource availability over time, there is a decrease in the number of ramets per genet on a local scale. This is similar to dynamics in stands of *Populus tremuloides* Michx. through time (Peterson and Squires, 1995).

In this study few significant differences in clonal size or structure were found by site type. This may have been a result of limited clone sample size, with many sample stems being identified as clonal ramets. Rogers (2000) also found only “modest” differences between upland and lowland. To more accurately assess site type differences, a more extensive sampling scheme would be necessary that can both identify the extent of clones on a local scale, but also cover a large area and capture a more significant number of clones. The fact that redwood genets can be very large and that there are no good surrogates for clone identification makes it difficult to economize on the sampling intensity.

*Conclusions*—In clonally spreading species many classical assumptions of how stands should be managed for biological diversity, maintenance of viable population size, regeneration, and site productivity need to be reevaluated. Our results indicate that only about one in six stems on these second-growth sites are distinct genets. This amounts to approximately a 16% reduction in effective population size relative to stem census size. Although in large populations such a reduction to effective population size is unlikely to have important consequences on genetic diversity, as populations become more restricted, the reduction of census size to effective population size becomes increasingly important. In redwood this is to some extent offset by its hexaploid genome, but it will still be important to have a better understanding of the effects of clonal spread on minimum population sizes needed to maintain existing genetic diversity. Our AFLP data do not allow us to make these estimates as allele frequencies for these dominant markers cannot be accurately estimated for a system that is not in Hardy-Weinberg equilibrium. As in the earlier study by Rogers (2000), we are unable to determine the extent of reproduction by seed. Single stem genets could be of seedling origin or be derived from a preexisting genet. Abnormalities in embryo development (Buchholz, 1939) and low levels of pollen production are believed to contribute to poor seed reproduction in redwood. However, in the long term, rare seedling recruitment may be sufficient to maintain levels of genetic diversity (Bond and Midgley, 2001). Understanding the relative proportions of seed and clonal reproduction will be crucial

to a better understanding of stand structure and evolution in redwood.

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